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Sørensen, Sara Nørgaard; Laurelle, Sacha; Engelbrekt, Christian; Baun, Anders

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# Short-term algal testing – a new approach for disclosing silver nanoparticle toxicity

Sara Nørgaard Sørensen<sup>\*1</sup>, Sacha Laurelle<sup>1</sup>, Christian Engelbrekt<sup>2</sup> and Anders Baun<sup>1</sup>

<sup>1</sup> Department of Environmental Engineering, Technical University of Denmark

<sup>2</sup> Department of Chemistry, Technical University of Denmark

\*sans@env.dtu.dk



## Introduction & objectives

Silver is the most common nanomaterial in commercially available products, used mainly for its antimicrobial properties.

In recent years, the environmental effects of silver nanoparticles (AgNPs) has been studied intensively, but still little is known about AgNP ecotoxicity and the underlying mechanisms.

A general challenge in aquatic toxicity testing of NPs is to control and describe the exposure (dose). Many processes affect NPs and causes the exposure dose to change during the exposure period.

The aim of this study is to:

- 1) Determine whether AgNP display ionic behaviour as measured by algal toxicity under various conditions
- 2) Investigate the effect of a shortened exposure period on AgNP and AgNO<sub>3</sub> toxicity

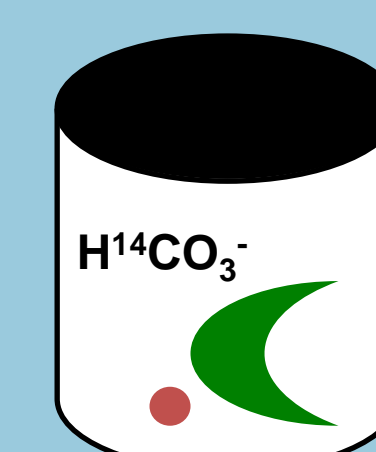
## Materials & Methods

Tested materials:

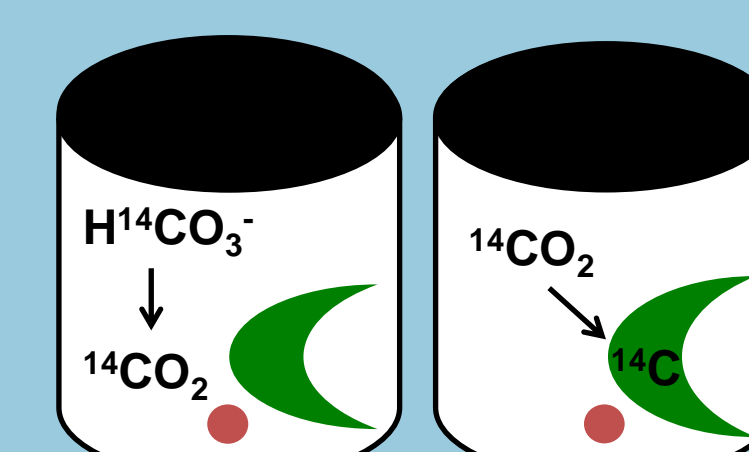
|                   |  |
|-------------------|--|
| AgNP-citrate      | Citrate coated AgNPs of nominal size 30 nm |
| NM-300K           | OECD reference AgNPs of nominal size 15 nm |
| AgNO <sub>3</sub> | Dissolved silver reference                 |

Algal tests with green algae *p. subcapitata*:

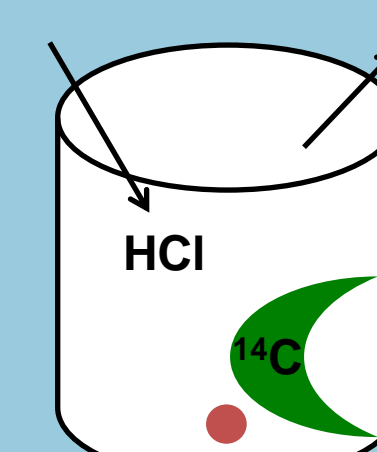
- |          |  |
|----------|--|
| 48h test | OECD 201 growth inhibition test                    |
| 2h test  | Newly developed 2h photosynthesis inhibition test: |



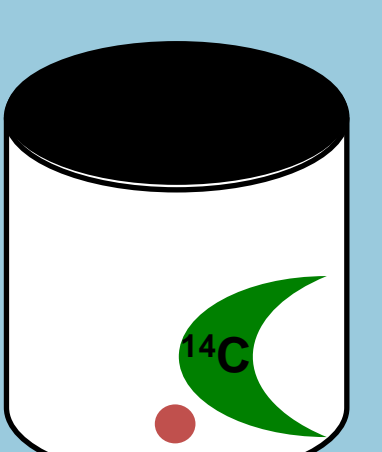
Test start  
Algae, NPs and H<sup>14</sup>CO<sub>3</sub> is added to media



2h exposure  
Via the carbonate system <sup>14</sup>CO<sub>2</sub> is formed and via photosynthesis the alga takes up <sup>14</sup>C from <sup>14</sup>CO<sub>2</sub>



Test end  
Added HCl stops photosynthesis and excess <sup>14</sup>CO<sub>2</sub> is evaporated



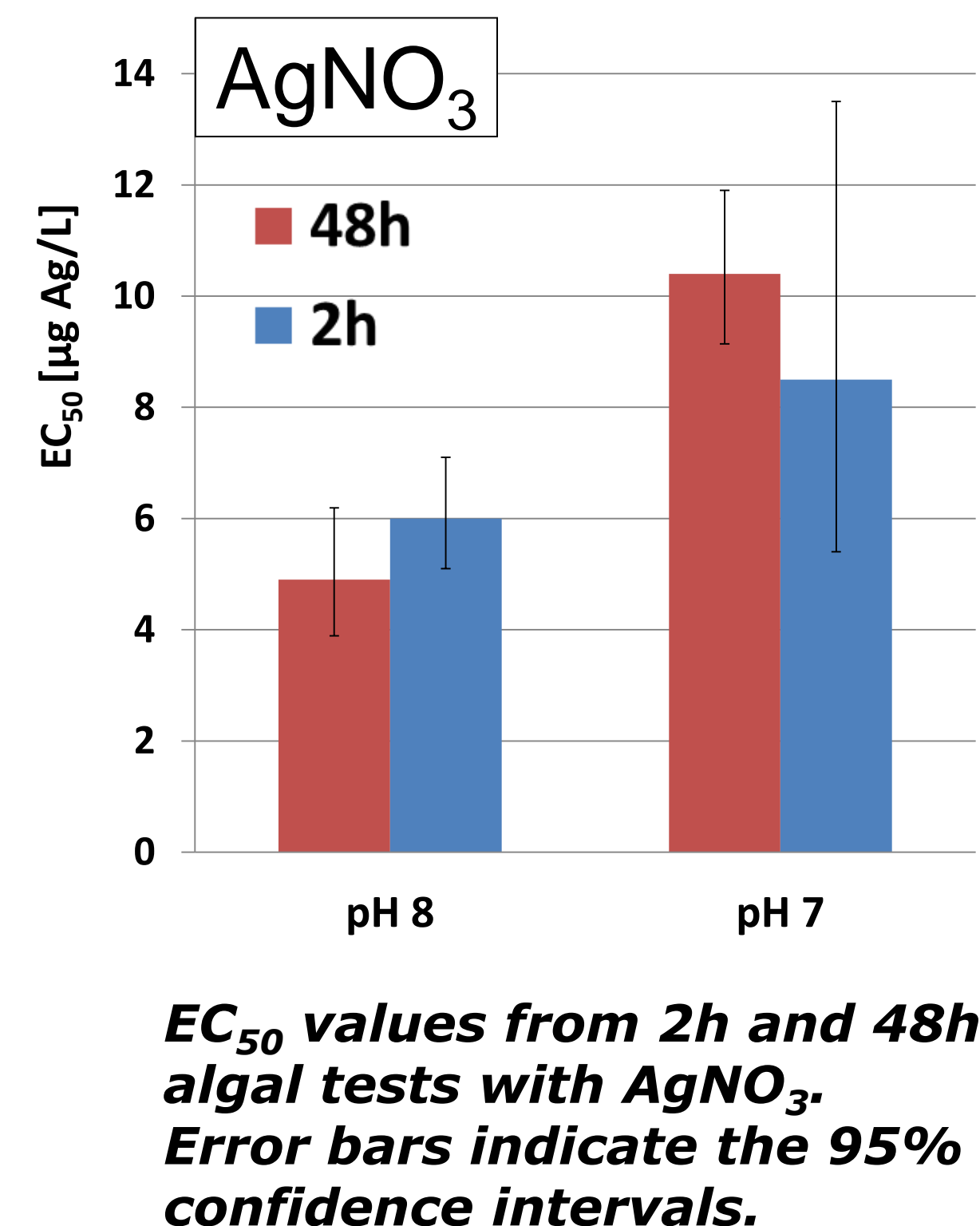
Analysis  
The <sup>14</sup>C amount is a measure for biomass and exposed algae is related to the control

## Results

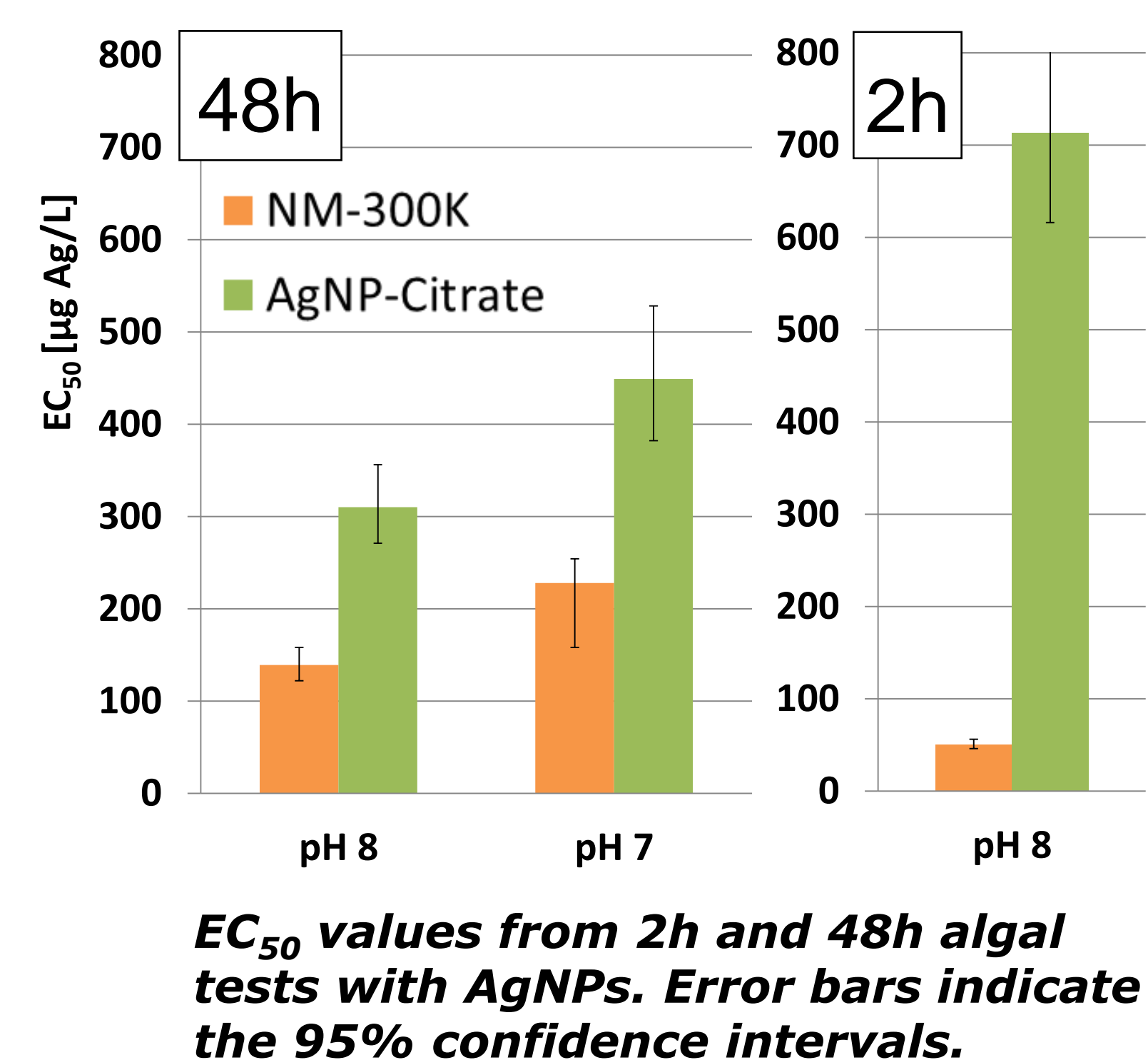
For AgNO<sub>3</sub> the EC<sub>50</sub> from 2h and 48h tests were not statistically significant different (p=0.05), meaning:

- The algal toxicity of dissolved silver occurs very rapidly
- The 2h test is applicable for testing toxicity of dissolved silver

The 2h test showed less precision at pH 7, due to interference with the <sup>14</sup>C-uptake from the carbon added as CO<sub>2</sub> in pH 7 tests.



## Results



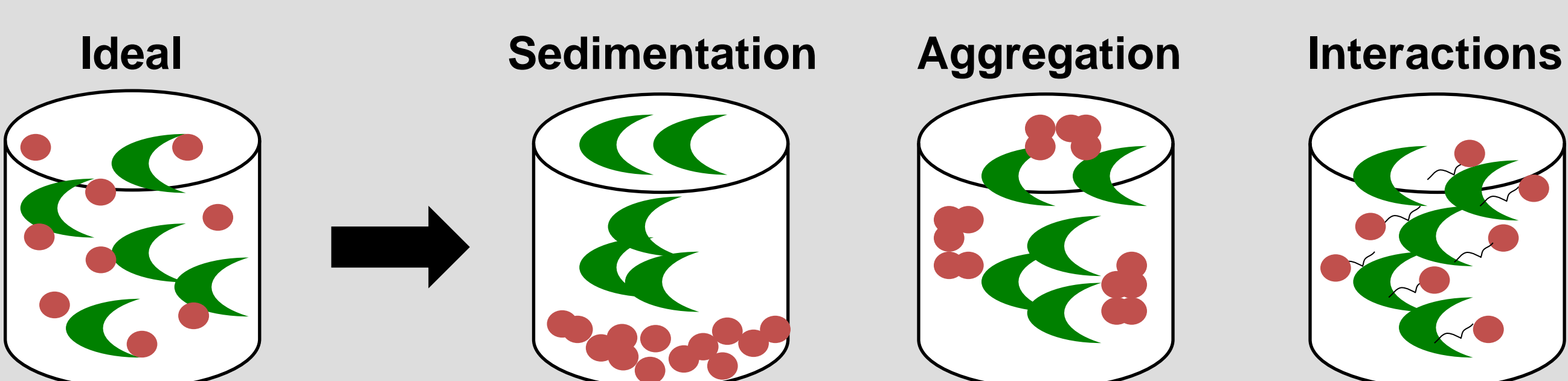
At pH 8, NM-300K was more toxic in the 2h test, while AgNP-citrate was less toxic - possibly due to the different test endpoints and different compositions of the AgNPs. NM-300K is smaller and has a greater ion release potential than AgNP-citrate.

The 2h test at pH 7, yielded poor dose-response relationships and no EC<sub>50</sub> could be calculated - possibly due to competition in <sup>14</sup>C-uptake with carbon from added CO<sub>2</sub>.

## Test duration: The shorter – the better ?

The 2h algal test setup provides a measure for photosynthesis inhibition. Moreover, it allows for high throughput screening of nanoparticle toxicity.

The reasoning for doing a very short exposure period (2h) is to minimize the potential confounding factors often experienced in standard algal tests with NPs due to time-dependant processes such as:



The aim is better control of the test system, more stable exposure conditions and thus improved prerequisites for obtaining dose-response relationships.

## Conclusions

- Overall, the changes in AgNP toxicity at various exposure conditions are in accordance with the expected outcome for ionic compounds
- AgNPs were less toxic than AgNO<sub>3</sub> based on total silver concentrations and the same order of toxicity was found in 2 and 48h tests: AgNO<sub>3</sub> > NM-300K > AgNP-citrate
- The 2h algal test was as sensitive as the 48h standard test to AgNO<sub>3</sub> toxicity at pH 8, demonstrating a fast toxic mechanism of dissolved silver in the algae *P. subcapitata*
- The 2h algal test setup provides a fast toxicity screening tool for AgNPs, and possibly other NPs – providing EC<sub>50</sub> values greater or lower than from 48h testing, depending on the characteristics of the AgNPs

